

The molecular mechanism for the *Tomato yellow leaf curl virus* resistance at the ty-5 locus

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV) is a major pathogen of tomato that causes extensive crop loss worldwide, including the US and Israel. Genetic resistance in the host plant is considered highly effective in the defense against viral infection in the field. Thus, the best way to reduce yield losses due to TYLCV is by breeding tomatoes resistant or tolerant to the virus. To date, only six major TYLCV-resistance loci, termed *Ty-1* to *Ty-6*, have been characterized and mapped to the tomato genome. Among tomato TYLCV-resistant lines containing these loci, we have identified a major recessive quantitative trait locus (QTL) that was mapped to chromosome 4 and designated *ty-5*. Recently, we identified the gene responsible for the TYLCV resistance at the *ty-5* locus as the tomato homolog of the gene encoding messenger RNA surveillance factor Pelota (Pelo). A single amino acid change in the protein is responsible for the resistant phenotype. Pelo is known to participate in the ribosome-recycling phase of protein biosynthesis. Our hypothesis was that the resistant allele of Pelo is a “loss-of-function” mutant, and inhibits or slows-down ribosome recycling. This will negatively affect viral (as well as host-plant) protein synthesis, which may result in slower infection progression. Hence we have proposed the following research objectives:

Aim 1: The effect of Pelota on translation of TYLCV proteins: The goal of this objective is to test the effect Pelota may or may not have upon translation of TYLCV proteins following infection of a resistant host.

Aim 2: Identify and characterize Pelota cellular localization and interaction with TYLCV proteins: The goal of this objective is to characterize the cellular localization of both Pelota alleles, the TYLCV-resistant and the susceptible allele, to see whether this localization changes following TYLCV infection, and to find out which TYLCV protein interacts with Pelota.

Our results demonstrate that upon TYLCV-infection the resistant allele of pelota has a negative effect on viral replication and RNA transcription. It is also shown that pelota interacts with the viral C1 protein, which is the only viral protein essential for TYLCV replication. Following subcellular localization of C1 and Pelota it was found that both protein localize to the same subcellular compartments.

This research is innovative and potentially transformative because the role of *Pelo* in plant virus resistance is novel, and understanding its mechanism will lay the foundation for designing new antiviral protection strategies that target translation of viral proteins.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Reviewed	0	1	1
Submitted	0	1	0

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
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Postdoctoral Fellow	Keren	Ido	State University of New York, Stony Brook	USA
M.Sc. Student	Avni	Ben	ARO	Israel

Contribution of Collaboration

All plasmid design and construction was performed by both labs. The experiments were designed, and results analyzed by both labs. The labs communicated regularly by mail and Skype conversations, and the American PI visited the Israeli lab once a year during the project.

ACHIEVEMENTS

Tomato yellow leaf curl virus (TYLCV) is a major pathogen of tomato (*Solanum lycopersicum*) worldwide. In many tomato-growing areas including Israel and the U.S, TYLCV has become the limiting factor for tomato production of both open-field and protected cultivation systems. TYLCV is a member of the genus *Begomovirus*, family *Geminiviridae*, of plant single-stranded (ss) DNA viruses. Begomoviruses are transmitted by members of the *B. tabaci* species complex in a persistent manner. As the whitefly vector, *Bemisia tabaci*, is widely distributed the introduction of TYLCV leads to rapid dissemination through commercial tomato crops with the likelihood of serious crop damage and economic losses. Prevention of TYLCV outbreaks is somewhat difficult to apply, the main control measures are careful monitoring and use of prophylactic measures, including insecticides to limit whitefly populations. Chemical control methods have been only partially effective, amid concerns about deleterious effects on the environment and potential for the vector to develop pesticide resistance.

Genetic resistance in the host plant is considered highly effective in the defense against viral infection in the field. This is especially true for those viruses that have prolific vectors which can rapidly produce very high populations in the field and are hard to contain. Thus, the best way to reduce yield losses due to TYLCV is by breeding tomatoes resistant or tolerant to the virus. Until now only six major TYLCV-resistance loci, termed *Ty-1* to *Ty-6*, have been characterized and mapped to the tomato genome using molecular DNA markers. We have shown that the TYLCV-resistant line TY172 contains a major recessive quantitative trait locus (QTL) that was mapped to chromosome 4 and designated *ty-5*. We also identified the gene responsible for the TYLCV-resistance at the *ty-5* locus - it was found that the tomato homolog of the messenger RNA surveillance factor Pelota (*Pelo*), controls the resistance at the *ty-5* locus. A single amino acid change in the protein seems to be in charge of the resistance phenotype. *Pelo* was recently implicated in the ribosome-recycling phase of protein biosynthesis.

Our hypothesis is that the resistant allele of *Pelo* is a “loss-of-function” mutant, and inhibits or slows-down ribosome recycling. This will negatively affect viral (as well as host-plant) protein synthesis, which may result in slower infection progression. Hence we have proposed the following research objectives:

Aim 1: The effect of Pelota on translation of TYLCV proteins: The goal of this objective is to test the effect *Pelota* may or may not have upon translation of TYLCV proteins following infection of a resistant host.

Aim 2: Identify and characterize Pelota cellular localization and interaction with TYLCV proteins: The goal of this objective is to characterize the cellular localization of both *Pelota* alleles,

the TYLCV-resistant and the susceptible allele, to see whether this localization changes following TYLCV infection, and to find out which TYLCV protein interacts with Pelota.

Aim 1. To test the effect Pelota may have on viral replication and transcription we have used two tomato near-isogenic lines (NIL) we have developed (Table 1) - a resistant line containing *ty-5* (Pelo-R, line 856), and a susceptible line (Pelo-S, line 857). Plants were inoculated with TYLCV using 50 viroliferous whiteflies per plant using clip cages. The plants were sampled at the indicated days post inoculation (DPI), total DNA and RNA were extracted, and viral DNA level as well as RNA corresponding to the different viral genes were determined using quantitative PCR. It was found that TYLCV DNA started to accumulate at 4-5 DPI in the susceptible line, and from 7 DPI onward viral DNA level went up sharply. In contrast, in the resistant line there is a clear delay of about a week in viral DNA accumulation level (Fig. 1).

Looking at transcription level of the complementary strand genes, C1 to C3 (as C4 is within the ORF of C1), all three gene show the same pattern of expression – earlier and higher transcription level in the susceptible plants and a clear delay in transcription start and inhibition of transcription level in the resistant plants (Figs 2-4). The same results were obtained when looking at the viral strand genes V1 and V2 – early and elevated transcription in the susceptible plants compares to the resistant plants (Figs 5, 6).

Aim 2. To test the interactions between the two alleles of pelota, i.e., the resistant allele (pelo-R) and the susceptible allele (pelo-S) and TYLCV proteins, we have used the yeast two-hybrid system (Y2H) followed by co-immunoprecipitation (co-IP). From the results presented in Table 2 we can see that both pelota alleles interacted with the viral C1 protein and with no other viral protein. Co-immunoprecipitation results (Fig. 7) indeed confirm the Y2H results – both alleles of Pelota are interacting with the virus C1 protein, but the susceptible Pelota allele binds C1 much stronger than the resistant allele (compare Fig. 1 lane 4 to lane 6). We have hypothesized that Pelota-R is a loss-of-function allele, and its reduced ability to interact with the viral C1 protein could partially account for its TYLCV-resistance ability.

Analysis of pelota *in planta* under TYLCV infection: plants of the TYLCV-resistant (Pelo-R) and susceptible (Pelo-S) NIL tomato lines described above were inoculated with TYLCV using whiteflies, while control plants were mock inoculated (treated with whiteflies without TYLCV). 21 days after inoculation the plant apex was harvested, total plant proteins extracted, and analyzed by Western blot using anti-pelota antibodies. Fig. 8 shows that after TYLCV-inoculation of both tomato lines there was a clear reduction in the amount of pelota. It seems that pelota was depleted from the inoculated leaves following inoculation, likely due to its interaction with the viral C1 protein, regardless of whether the plant contained the resistant or the susceptible allele of pelota.

Pelota localization in planta using confocal imaging: Pelota-R and Pelota-S fused to mCherry were transiently expressed in *Nicotiana benthamiana* (NB) plants using *Agrobacterium*. Cherry fluorescence was examined in epidermal cells at the lower face of leaves 48 hours post injection (Fig. 9). In a second experiment, we have compared the cellular localization of pelota to calreticulin, which serves as an endoplasmic reticulum (ER) marker, fused to GFP (Fig. 10). From the results shown in Figures 9 and 10, we can conclude that both alleles of pelota, the resistant and the susceptible, have similar cellular localization patterns. Both alleles are largely cytosolic, show cytoplasmic strands and are also in the nucleus. Based on the marker for the ER-lumen, pelota does not co-localize with the ER. As our results indicate that pelota and TYLCV C1 protein interact, we fused C1 to GFP and compared the cellular localization of C1 to that of both pelota alleles. It was found that C1 is mainly localizes to the nucleus, but can be found in the cytoplasm and is nearly identical to the cellular localization of both Pelota alleles (Fig. 11).

TYLCV C1 gene encodes Rep (replication-associated protein) which is a multifunctional protein involved in viral replication and transcriptional regulation. This is the only viral protein absolutely required for viral replication. Hence, it is not surprising that the TYLCV-resistance gene Pelota “targets” the viral C1 protein. Our results indicate that Pelota interacts with the viral C1 protein, that the susceptible allele of pelota binds C1 better than the resistant allele, and that both proteins – C1 and Pelota - can be found in the same subcellular localizations. It seems that the interaction C1-Pelota is essential for viral replication and transcription, and that the resistant allele doesn’t interact as it should with the viral C1, hence the reduction in viral replication and transcription which results in the resistant phenotype. This supports our basic assumption that the resistant allele of pelota is a “loss-of-function” allele. To the best of our knowledge this is the first demonstration that in plants pelota is essential for TYLCV infection, and that it interacts with the viral C1 protein.

Finally, we laid a foundation for upcoming studies of regulation of the Pelota gene by adapting in planta and in vitro systems for studies of epigenetic regulation of plant gene expression; specifically, we characterized the effects of plant histone deubiquitinases OTLD1 and OTU1 on their target chromatin.

Scientific and agricultural implications. Collectively, our data explain how the combination of *Ty-1/Ty-3* with *ty-5* can enhance TYLCV resistance, as both resistances operate in a different manner – *Ty-1/Ty-3* works *via* gene silencing while *ty-5* through hindering pelota-C1 interactions. Understanding how different resistances operates will help design new strategies for viral resistance and inform how and which resistance genes should be combined to breed for plants with enhanced disease resistance.

Changes to Original Research Plan

In essence no changes were made to the original Research Plan, except that more emphasis was placed on viral replication and gene transcription.

Publications for Project IS-4953-16

Stat us	Type	Authors	Title	Journal	Vol:pg Year	Cou n
Published	Reviewed	<i>Keren I., Lapidot, M., Citovsky, V</i>	Coordinate activation of a target gene by KDM1C histone demethylase and OTLD1 histone deubiquitinase in Arabidopsis	<i>Epigenetic's</i>	14 : 602-610 2019	Joint
Published	Reviewed	<i>Keren, I., Lacroix, B., Kohrman, A., & Citovsky, V</i>	Histone deubiquitinase OTU1 epigenetically regulates DA1 and DA2, which control Arabidopsis seed and organ size	<i>iScience</i>	23 : 100948 2020	US only
Submitted	Reviewed				:	Joint

Appendix

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Unpublished data:

Tables

Figures

Published papers

Unpublished data

Tables

Table 1. *Pelo* near isogenic lines

Line	Genotype	TYLCV
PRT-856	<i>ty-5/ty-5</i>	Resistant
PRT-857	+/+	Susceptible

Table 2. Interactions between the pelota R and S alleles and TYLCV proteins as tested by Y2H system

Viral gene	Pelota-S		Pelota-R	
	X-gal	Leu	X-gal	Leu
TY-V1	-	-	-	-
TY-V2	-	-	-	-
TY-C1	+	1:100	++	1:1000
TY-C3	-	-	-	-
TY-C4	-	-	-	-

The coding region of the pelota alleles [from TYLCV-susceptible (S) and resistant (R) plants] was amplified by PCR and cloned in the ‘bait’ plasmid pEG202 and in the ‘prey’ plasmid pJG4-5. The viral genes were amplified and cloned in the same way. All the constructed vectors were confirmed by sequencing. The different constructs were introduced into the EGY48 yeast strain together with the pSH18.34 vector (OriGene) carrying the URA3 selectable marker and the *lacZ* reporter gene fused to eight *lexA* operators. Yeast transformants were selected on plates containing complete minimal (CM) dropout medium without uracil and histidine but with Glc as a unique carbon source. Individual yeast colonies were tested for expression of β -galactosidase by using X-gal and by their ability to grow on plates containing CM dropout medium without uracil, histidine, and leucine. Due to technical problems, we were unable to test the interaction with the viral C2 protein, since C2 gave a non-specific positive reaction (false-positive) in Y2H.

Figures

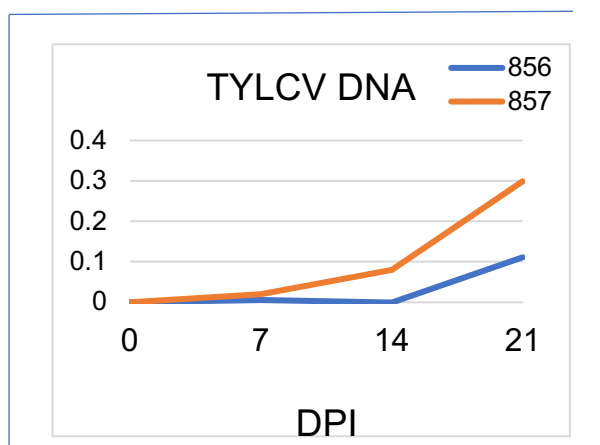


Figure 1. Accumulation of TYLCV total DNA in tomato plants apex, using two NIL's, a resistant (856) and a susceptible (857) line. Plants were inoculated with TYLCV using 50 viroliferous whiteflies per plant using clip cages. The whiteflies were given a 48hr transmission access period, at the end of which the clip cages were removed, the plants were sprayed and kept in an insect-proof greenhouse. The plants were sampled at the indicated days post inoculation (DPI), total DNA was extracted, and viral DNA level was determined using quantitative PCR. Each time point is an average of five different plants. Each experiment was repeated twice.

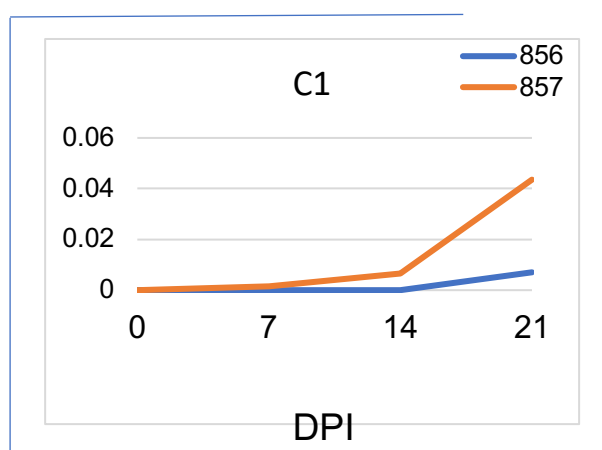


Figure 2. Transcription level of TYLCV C1 gene. Plants were inoculated with TYLCV as described in Figure 1. The plants were sampled at the indicated days post inoculation (DPI), total RNA were extracted, and level of viral RNA corresponding to the different viral genes was determined using quantitative PCR. Each time point is an average of five different plants. Each experiment was repeated at least twice.

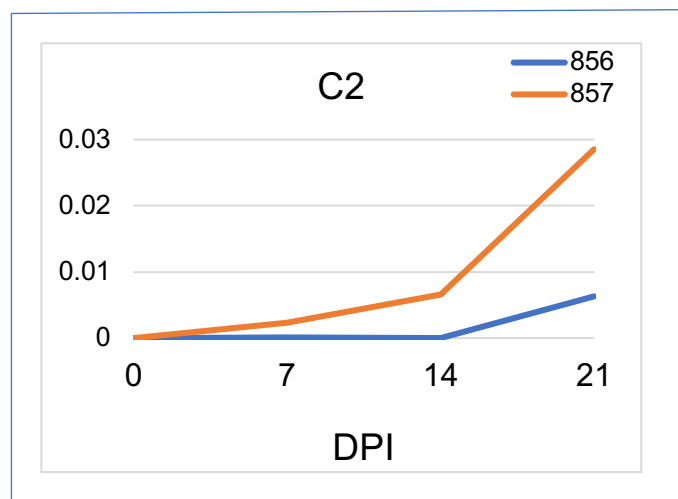


Figure 3. Transcription level of TYLCV C2 gene. The same experiments described above were also used to quantitate the effect Pelo may have on the transcription level of the different viral genes.

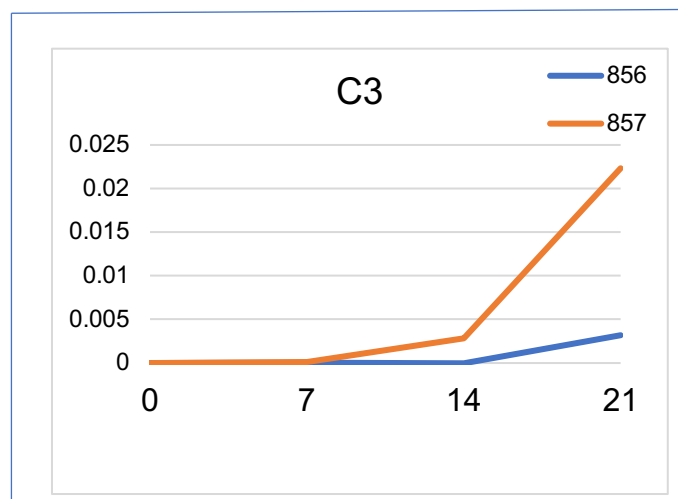


Figure 4. Transcription level of TYLCV C3 gene.

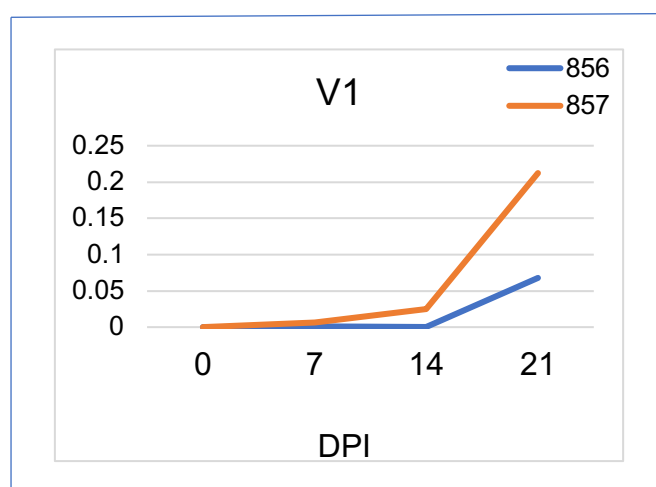


Figure 5. Transcription level of TYLCV V1 gene.

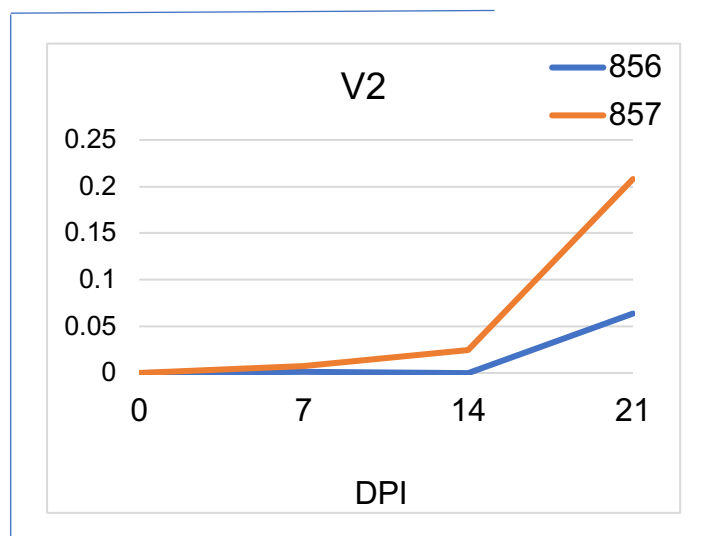


Figure 6. Transcription level of TYLCV V2 gene.

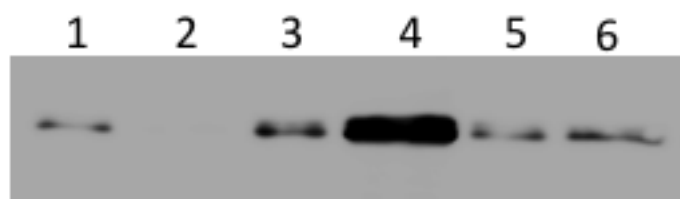


Figure 7. Co-immunoprecipitation *in planta*. Lanes: (1) Column input of free mCherry+C1:HA; (2) Immunoprecipitation (column elution) of free mCherry+C1:HA; (3) Column input of Pelota-S:mCherry+C1:HA; (4) Immunoprecipitation (column elution) of Pelota-S:mCherry+C1:HA; (5) Column input of Pelota-R:mCherry+C1:HA; (6) Immunoprecipitation (column elution) of Pelota-R:mCherry+C1:HA.

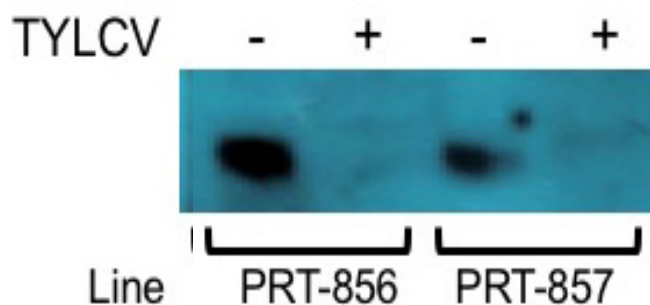


Figure 8. Western blot analysis of total tomato protein reacted with anti-pelota antibodies. Top: Plants inoculated with TYLCV are marked +, non-inoculated control plants marked -.

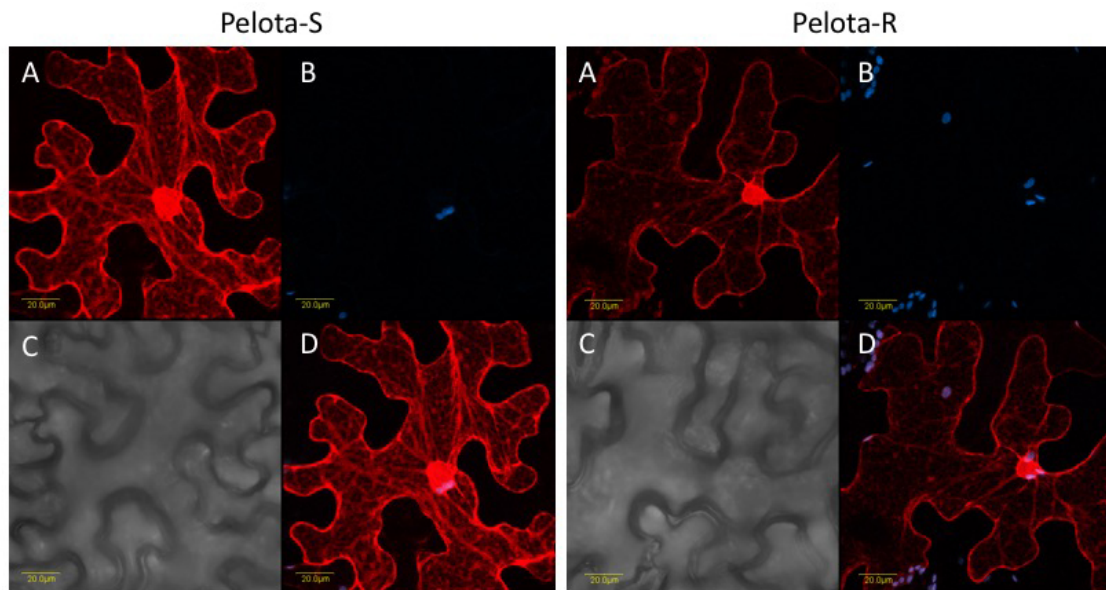


Figure 9. Cellular localization of pelota-S and pelota-R fused to mCherry. Left panel: Pelota-S. A: pelota-S fused to mCherry. B: chlorophyll auto-fluorescence. C: Light microscopy (DIC). D: A and B superimposed. Right panel: Pelota-R. A: pelota-R fused to mCherry. B: chlorophyll auto-fluorescence. C: Light microscopy (DIC). D: A and B superimposed.

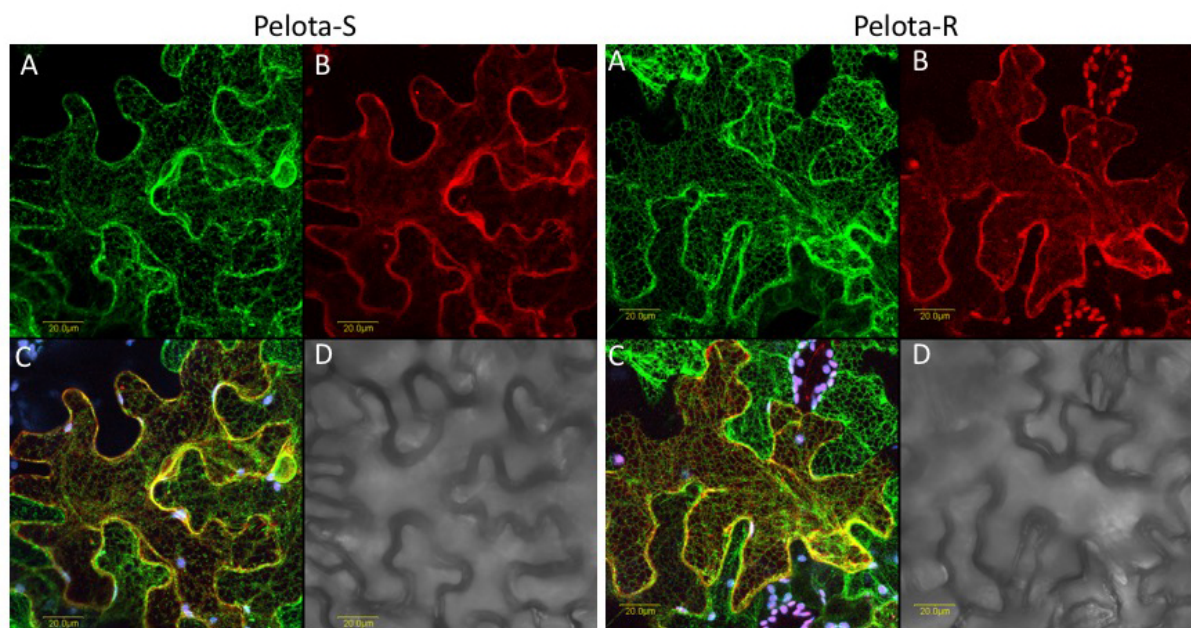


Figure 10. Cellular localization of pelota tagged with mCherry compared to localization of calreticulin tagged with GFP. Left panel: Pelota-S. A: calreticulin fused to GFP. B: pelota-S fused to mCherry. C: A and B superimposed. D: Light microscopy (DIC). Right panel: Pelota-R. A: calreticulin fused to GFP. B: pelota-S fused to mCherry. C: A and B superimposed. D: Light microscopy (DIC).

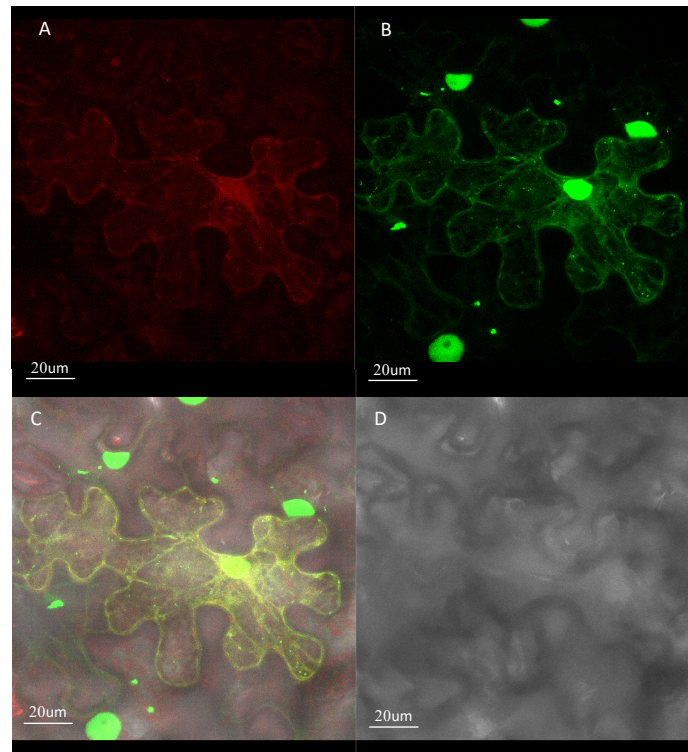


Figure 11. Cellular localization of Pelota-R tagged with mCherry compared to localization of TYLCV-C1 tagged with GFP. A: Pelota-R fused to mCherry. B: TYLCV-C1 fused to GFP. C: A and B superimposed. D: Light microscopy (DIC).